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Drug repurposing for antimicrobial activity on selected bacterial species

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Abstract

Increasing cases of multidrug-resistant pathogens have evolved into a global health crisis. *Salmonella sp., Proteus sp.* and some strains of *Bacillus* especially isolated from soil are pathogenic and harmful to human health. Antibiotics are usually used in the treatment of selected bacterial species are fluoroquinolones (Ciprofloxacin, Azithromycin and Cephalosporins) for *Salmonella species*, Clindamycin, Vancomycin, Cephalosporins and Penicillins are effective against *Bacillus* infections and Nitrofurantoin, Tetracycline, Polymyxins, Ceftriaxone, Quinolone, Gentamicin (plus Ampicillin) are used in the management of *Proteus species*. These bacteria are also associated with antibiotic resistance, and infections caused by pathogens result in high mortality and morbidity. Thus there is a need of drug repurposing and our study gives the choice of drug for effective treatment by repurposing already known and tested drugs against the bacterial infections. In present study four antibiotics (Azithromycin, Ciprofloxacin, Doxycycline, Ofloxacin), one anti-malarial (Quinine) and one anthelmintic (Albendzole) agents were screened for repurposing of antibacterial activity on three different species of microorganisms i.e. *Salmonella sp., Proteus sp.* and *Bacillus sp.* The multiple drug resistance of *Salmonella sp., Proteus sp.* and *Bacillus sp.* against tested drug concentrations was evaluated by using disk diffusion method. Antimicrobial activity was confirmed by the presence of zone of inhibition. The test results indicated that Ciprofloxacin and Doxycycline worked on all the three selected species. It was also found that Quinine and Albendzole were effective against *Salmonella sp.*, and *Proteus sp.* After that we selected different ratio of these two drugs and found that Ciprofloxacin with Doxycycline at 1:1 proportion gave best zone of inhibition for all the three bacterial species that was 30 mm, 35 mm and 37 mm, respectively than the individual activity of each drug. Thus, our study emphasizes on the easy repurposing of available antimicrobial agents which will be cost effective and less time consuming.

Keywords: Drug repurposing, Antimicrobial activity, Antibiotics.

Introduction

Drug repurposing is a secure pathway to speed up successful development of the novel drug. This process uses either FDA (Food and Drug Administration) approved drugs or drugs that are failed in clinical trials. These drugs have its detailed information on formulation, potential toxicity and

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pharmacology. Due to scientific advancements in the biological sciences, informatics and genomics, it is possible to identify secondary response of existing drugs in drug repurposing. Drug repurposing have significant role within the finding of various possible antibiotics. Microbial infection like bacteria emerged as a growing public health threat by multidrug resistance for current drugs at worldwide level. This increases the urgent need of improvement of new antibiotics that may successfully fight multidrug-resistant bacterial infections (MDRBIs) (1).

Different Institutions identified that antimicrobial resistance is a worldwide health risk that has compounded through the reduction in the discovery and improvement of new antimicrobial agents (2,3). Consequently, the development of recent antimicrobial therapeutic strategies requires immediate attention to keep away from the ten million deaths predicted to occur by 2050 because of multidrug-resistant (MDR) bacteria. Despite the increasing interest in the improvement of repurposing drugs, only few repurposing drugs are below scientific improvement towards Gram-negative pathogens (4).

Drug repositioning also termed in means by their motif such as restoring, re-profiling, therapeutic switching, re-purposing and its side activities. As the existing drug have gone through testing procedures, this shortcut method of re-utilizing can reduce the cost, time, risk and various known parameters in concern. Archived drug that failed to step into market but have safety and risk clearance have also shown potential to repurpose (5,6).

Material and Method

Isolation and identification: Bacterial species isolated from soil sample were biochemically tested and identified recommended in the Bergey's Manual of Determinative Bacteriology (7). Then these strains were maintained by incubation at specific time and temperature on growth media for *in-vitro* investigation in our institutional laboratory. Further commercially available antibiotic disk diffusion test was performed to detect sensitiveness, intermediate inhibitory zone and resistance. This was detected using following antibiotics - Tetracyclin, Gentamycin, Ampicillin, Co-trimoxazole, Cefuroxime, Ofloxacin, Erythromycin, Oxaclin, Cefaclor via disc diffusion method. All the drug resistant strains were then grown at 37^0C (98.6^0F) in nutrient broth and subcultures were made freshly to run subsequent experiments. Proper sterile conditions were maintained and good laboratory procedures were followed while dealing with these multidrug microbial strains.

In the present study four antibiotics (Azithromycin, Ciprofloxacin, Doxycycline, Ofloxacin), one anti-malarial (Quinine) and one anti-helmintic (Albendzole) agents were screened for repurposing of antibacterial activity on three different species of microorganisms i.e. *Salmonella sp., Proteus sp.* and *Bacillus sp.* (8).

Antimicrobial sensitivity test: Incubation procedure - Under aseptic condition using sterile swab dip into broth medium of test organisms (target bacterial strains) then gentle pressing of swab end against

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the inside wall of tube to decant excess soaked liquid, bacterial lawn on Muller-Hinton agar plate was prepared by swabbing in uniform manner in all direction.

Using agar wells diffusion technique wells were punched with diameter of 2 mm in the previously inoculated test bacterial culture on Muller Minton agar plate. Minimum Inhibition Concentration (MIC) was determined by micro-dilution of sample (test) drug in sequential decreasing order of product concentration (100, 50, 25 and 12.5ug/ml). Observations were made after incubation at 37^0C for 20hrs.

Visible inhibitory growth of lowest concentration of test drugs (MIC) was determined by measuring the diameter using ruler template for zone of inhibition (8).

Combination of Drug for repurposing screen assay: Multiple drug agents showed improved effect when used together against antibiotic resistant bacteria (9). Well diffusion method was used for this screen assay (8). Mechanism underlying in this event maybe based on the chemical interaction, inhibitory action with dual force or other unknown factors.

Micro-dilution was done in two ratios (1:1 & 1:2) with reciprocal dilution by using minimum inhibition concentration of test drugs previously studied and measured value estimated.

Combined effect on varied antimicrobial induction was done on the test microorganisms with freshly broth culture incubated at 37^0C for 24 hrs, plated on Muller Hinton Agar and the result was measured by the zone of inhibition dimension. Comparative study of single drug and two drugs effect was done to detect the likelihood of synergism.

Result

The antimicrobial activity was assessed following standardized method. The manual estimation of zone of inhibition by measuring the diameter circling the clear zone is easy and feasible. Comparative antimicrobial activity of antibacterial analysis has done for Azithromycin, Ciprofloxacin, Ofloxacin, Doxycycline, Quinine (anti-malarial drug) and Albendazole (anthelminthic drug) against selected pathogens. Table 1 shows standardization of above drug concentration against the different species of test bacteria.

Table 1: Standard concentration of drugs used in the present study against the test pathogens:

Test Bacterial Strain	Azithromycin (µg / ml)	Ciprofloxacin (µg / ml)	Doxycycline (µg / ml)	Ofloxacin (µg / m l)	Quinine (µg / ml)	Albendzole (µg / ml)
Salmonella sp.	25	12.5	25	12.5	25	200
Proteus sp.	-	12.5	100	12.5	12.5	-
Bacillus sp.	-	12.5	100	12.5	-	-

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Combined effect of two different drugs was tested against bacterial strain that showed resistance against other choice of antibiotics. Individual minimum inhibitory concentration towards test drug was compared with collective drug result in which we have found that ciprofloxacin with doxycycline at 1:1 proportion gave best inhibition zone for all the three selected bacterial species that was 30 mm, 35 mm and 37 mm, respectively. Thus it was concluded that ciprofloxacin in combination with doxycycline increased antibacterial activity against tested Salmonella *sp.*, *Proteus sp.* and *Bacillus sp.* (Table 2).

Table 2: Minimum inhibitory concentration of different antibiotics (individual & combined) used in the present study:

⬇ ➡	**Name of Bacterial species**	***Salmonella sp.***	***Proteus sp.***	***Bacillus sp.***
S.No	**Name of Drug**	**Zone of Inhibition (in mm)**		
1.	**Azithromycin**	**15**	**-**	**-**
2.	**Ciprofloxacin**	**32**	**32**	**34**
3.	**Doxycycline**	**15**	**18**	**17**
4.	**Ofloxacin**	**21**	**18**	**-**
5.	**Quinine**	**16**	**14**	**-**
6.	**Albendzole**	**21**	**-**	**-**
7.	**Ciprofloxacin + Doxycycline (1:1)**	**30**	**35**	**37**
8.	**Ciprofloxacin + Doxycycline (1:2)**	**26**	**34**	**37**
9.	**Doxycycline + Ciprofloxacin (1:2)**	**30**	**34**	**36**

Discussion

Antibiotics are the important agents in combating microorganism infections. Drugs play a major role within the interference and treatment of human diseases. The microorganisms like bacteria have the genetic ability to spread and gain resistance to synthetic drugs that are used as therapeutic agents.

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Despite ongoing efforts to identify new drugs or alternatives to antibiotics, no new classes of antibiotic or their alternatives have been clinically approved in the last three decades. A combination of antibiotics and non-antibiotic compounds that could inhibit bacterial resistance determinants or enhance antibiotic activity offers a sustainable and effective strategy to meet multidrug-resistant bacteria (10-12). Drug-drug interactions can be divided into three types: synergy; no interaction; and antagonism (13).

In the present study, efforts have been taken to find out any kind of positive interaction among the common antibiotics which may cover the pathway for repurposing of these drugs against the drug resistant pathogens which cannot be treated by following the conventional treatment strategy. Ciprofloxacin and Ofloxacin are known to cause damage in bacterial cell through DNA manipulating pathways. Whereas anti-bacterial drug Azithromycin and Doxycycline also known remedy for malarial infections. The definite mechanism of quinine is still not discovered yet. Azithromycin and anti-parasitic agent Albendazole gave better results for Salmonella species. All the three selected bacterial strains of *Salmonella species, Proteus sp.* and *Bacillus sp.* showed sensitivity against Doxycyline and Ciprofloxacin. These antibacterial medicines are counted among the world bestselling drugs. But the generation of multidrug resistance into existence is one of the top most threats to human kind. The pace of adapting for defense mechanism by the microbial community is spontaneous and unpredictable. The outcome obtained in the present study indicated that how the drugs included in the study can show activity on other microbes also for which they are not normally prescribed. Our findings were found according to various other similar type of studies (14-17). The synergistic action of two drugs Ciprofloxacin and Doxycycline was reported by the increased zone of inhibition as compared to their individual action against the tested pathogens. Thus the results indicated positive aspect of using these drugs in combination in different ratios and also paved a way to repurposing treatment process using these drugs.

Conclusion

The use of antibiotics for treating any kind of infection or disease is very common these days. Their use often goes unmonitored or uncontrolled. However, current antibiotics became less effective as a result of the emergence of drug-resistant microorganisms. It is imperative to research newer drugs that are active against drug resistant microorganisms. The drug repurposing results in quicker, cost effective and easy approach to deal with the problem by giving new useful roles to the already known drugs against a different microbial community. The present study was a successful trial for the same and the results are found to be very optimistic in drug repurposing field.

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Author's contributions

The entire authors have contributed equally.

Conflict of interests

There is no conflict of interest regarding publication of this article.

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